

Prevalence and Distribution of *Sarcocystis* in Buffaloes and Sheep in Egypt

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Abstract

Sarcocystis spp. is cyst forming protozoa that contains more than 200 species and belong to the phylum *Apicomplexa*. This study aimed to investigate the prevalence of *Sarcocystis* spp. in buffalo and sheep carcasses slaughtered in Egypt macroscopically and microscopically. For this purpose, a total of 400 buffalo and sheep carcasses were examined at Tanta abattoir, Egypt for the detection of *Sarcocystis* spp. from July 2020 to June 2021. The results revealed that the prevalence of macroscopic sarcocysts was 26.5% in slaughtered buffaloes and 0% in slaughtered sheep, while the prevalence of microscopic sarcocysts was 56% in slaughtered buffaloes and 80.5% in slaughtered sheep. The prevalence of sarcocysts in old buffaloes and sheep was higher than in young buffaloes and sheep. The most affected organs with microscopic sarcocysts were the oesophagus followed by the tongue, masseter muscle, skeletal muscles and finally heart. The obtained results confirmed that the examined buffaloes and sheep are infected with *Sarcocystis* species due to the abundance of final hosts, especially dogs and cats that encourage the spreading of infection by this protozoan parasite. Therefore, efficient cooking of buffalo meat is highly recommended before serving to humans.

KEYWORDS

Sarcocystis, Meat, offals, buffaloes, sheep, Egypt.

INTRODUCTION

Buffaloes (*Bubalus bubalis*) are reared in Middle Eastern countries, particularly in Egypt for the purpose of meat and milk production. Buffaloes' meat can provide humans with part of their needs of essential amino acids, minerals, and vitamins. However, buffaloes' meat might act as a considerable source for transmission of foodborne pathogens to humans such as several bacterial and parasitic species.

Sarcocystosis is a parasitic zoonosis distributed all over the world caused by *Sarcocystis* species which are apicomplexan parasites requiring intermediate and definitive hosts to complete their life cycle, inside the intermediate host occurs the asexual life cycle with cysts formation called *Sarcocystis*, while the sexual life cycle occurs inside the definitive host. Final hosts including carnivores and humans usually become infected by ingestion of undercooked meat containing *Sarcocystis*, while intermediate hosts become infected by ingestion of sporulated oocysts or sporocysts in contaminated food or water (Dubey, 2015).

Buffaloes are intermediate hosts for some species of *Sarcocystis*; they may harbour macroscopic or microscopic *Sarcocystis* in their striated muscles (Dubey and Lindsay, 2006). Buffaloes (*Bubalus bubalis*) are natural intermediate hosts for four species of *Sarcocystis* including *S. fusiformis* and *S. buffalonis* with felids as the final host, *S. levinei* with canids as the final host and *S. dubeyi* with an unidentified final host (Hilali *et al.*, 2011).

Sheep act as intermediate hosts for 6 *Sarcocystis* species including *S. gigantea*, *S. medusififormis*, *S. tenella*, *S. arieticanis*, *S. microps*, and *S. mihoensis*, which can be morphologically differentiated by variations in the ultrastructure of the sarcocyst wall. Sarcocysts of *S. tenella* and *S. arieticanis* are microscopic sarcocysts and transmitted by canine final hosts, whereas *S. gigantea* and *S. medusififormis* form macroscopic sarcocysts and are transmitted by cats (Dubey *et al.*, 2015).

The genus *Sarcocystis* contains more than 200 species characterized by a worldwide geographic distribution. Three species of *Sarcocystis* including *S. hominis*, *S. heydorni*, and *S. sui hominis* are known to use humans as the final host. Humans become infected by ingesting sarcocysts in muscular tissues of intermediate hosts, while the intermediate hosts are infected by ingesting sporulated oocysts or sporocysts in contaminated food or water. Two different clinical forms of sarcocystosis in humans: an intestinal form, caused by *S. hominis*, *S. heydorni* and *S. sui hominis*, and a muscular form, caused by *S. nesbitti*, the only *Sarcocystis* species that uses humans as intermediate host (Dubey, 2015).

In Egypt, buffaloes' meat is a major meat supply in the Egyptian market where males are only slaughtered, and there is a strict law prohibits slaughtering of female buffaloes unless become out of the production cycle. The prevalence of *Sarcocystis* spp. in buffaloes' meat of both sexes in Egypt is less investigated.

Moreover, mutton represents an essential source of animal protein for human and contributes considerably in the Egyptian

food security.

In sight of the previous facts, the present study was conducted to investigate the prevalence of sarcocysts in slaughtered buffaloes and sheep macroscopically and microscopically. Besides, some associated risk factors (age and sex) were assessed. Additionally, organ distribution of microscopic sarcocysts in slaughtered buffaloes and sheep using was discussed.

MATERIALS AND METHODS

This study was conducted according to the guidelines of Benha University for the use of animals, where no living animals were used in the current investigation.

Study animals

A total number of 200 slaughtered buffaloes (100 young males and 100 old females) and 200 slaughtered sheep (100 young males and 100 old females) were examined for detection of macroscopic and microscopic sarcocysts during the period from July 2020 to June 2021 at Tanta abattoir, Egypt. Each animal was identified by sex (male-female) and by age (young-old). The age of investigated animals was assessed by visual inspection of teeth.

Gross examination

Macroscopic sarcocysts were identified by visual inspection of muscular tissues according to Huong (1999). Muscle masses from the oesophagus, heart, tongue, masseter muscle and skeletal muscle were sliced to facilitate gross inspection to detect any macroscopic sarcocysts in slaughtered buffaloes and sheep. Also, the percentage of positive and negative macroscopic sarcocysts was recorded for both species.

Samples collection

Fresh samples from the oesophagus, tongue, heart, masseter muscle and skeletal muscle were collected from slaughtered buffaloes and sheep to determine the prevalence of microscopic sarcocysts. All samples were labelled and immediately fixed in 10% neutral buffered formalin for further histological examination at Department of Pathology, Faculty of Veterinary Medicine, Kafrelshiekh University, Egypt.

Histological examination

Samples were fixed in 10% neutral buffered formalin and processed for histological technique according to Bancroft and Gamble (2008) through dehydration in graded ethanol, embedded in paraffin wax, sectioned at 5 µm in thickness and stained by Hematoxylin and Eosin (H&E). The slides were visually screened for microscopic sarcocysts by highly experienced staff at Department of Pathology, Faculty of Veterinary Medicine, Kafrelshiekh University. All results were photographed by a digital camera.

RESULTS

A total of 200 buffalo carcasses and 200 sheep carcasses were examined in Tanta abattoir, Egypt for detection of sarcocysts during the period from July 2020 to June 2021. The results in Table 1, revealed that the prevalence of macroscopic sarcocysts was 26.5% in slaughtered buffaloes (42% in females and 11% in males) and 0% in slaughtered sheep, while the results in Table 2, showed that the prevalence of microscopic sarcocysts was 56%

in slaughtered buffaloes (79% in females and 33% in males) and 80.5% in slaughtered sheep (93% in females and 68% in males). Moreover, the results in Tables 1 and 2, revealed a higher prevalence of sarcocysts in old buffaloes (42% macroscopic sarcocysts and 79% microscopic sarcocysts) than in young buffaloes (11% macroscopic sarcocysts and 33% microscopic sarcocysts) and a higher prevalence of microscopic sarcocysts in old sheep (93%) than in young sheep (68%).

Table 1. The prevalence of macroscopic sarcocysts in slaughtered buffaloes and sheep concerning age and sex.

Species	Age Group	Sex	Examined Number	Positive Number	Prevalence %
Buffaloes	1-3 years	Male	100	11	11%
	5-8 years	Female	100	42	42%
Total			200	53	26.50%
Sheep	1-2 years	Male	100	0	0%
	3-5 years	Female	100	0	0%
Total			200	0	0%



Fig. 1. Macroscopic appearance of sarcocysts isolated from an infected buffalo's oesophagus.

Table 2. The prevalence of microscopic sarcocysts in slaughtered buffaloes and sheep concerning age and sex.

Species	Age Group	Sex	Examined Number	Positive Number	Prevalence %
Buffaloes	1-3 years	Male	100	33	33%
	5-8 years	Female	100	79	79%
Total			200	112	56%
Sheep	1-2 years	Male	100	68	68%
	3-5 years	Female	100	93	93%
Total			200	161	80.50%

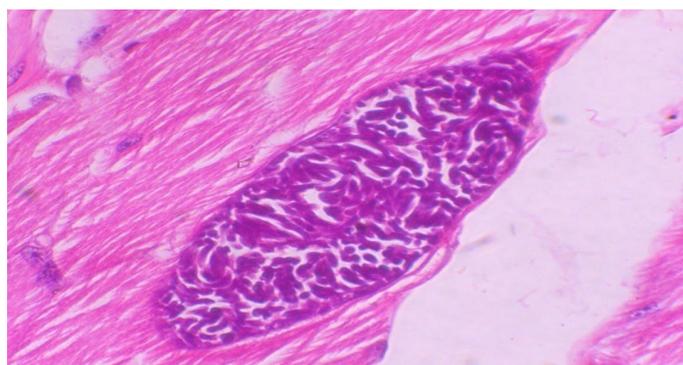


Fig. 2. Histological section representing a microscopic sarcocyst isolated from an infected buffalo's tongue. The sarcocyst initiated no tissue reaction (H&E stain).

The results in Tables 3 and 4, demonstrated that the most affected organs with microscopic sarcocysts were the oesophagus (53.5% in buffaloes and 76% in sheep) followed by the tongue (45.5% in buffaloes and 70% in sheep), masseter muscle (41% in buffaloes and 53% in sheep), skeletal muscles (32% in buffaloes and 48.5% in sheep) and finally the heart (24% in buffaloes and 38.5% in sheep).

Table 3. Organ distribution of microscopic sarcocysts in slaughtered buffaloes.

Organ	Examined Number	Positive Number	Prevalence %
Oesophagus	200	107	53.50%
Heart	200	48	24%
Tongue	200	91	45.50%
Masseter muscle	200	82	41%
Skeletal muscle	200	64	32%

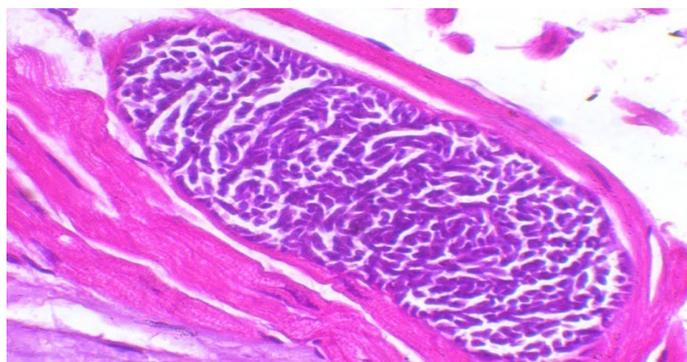


Fig. 3. Histological section representing a microscopic sarcocyst in the skeletal muscles isolated from an infected buffalo. The sarcocyst initiated no tissue reaction (H&E stain).

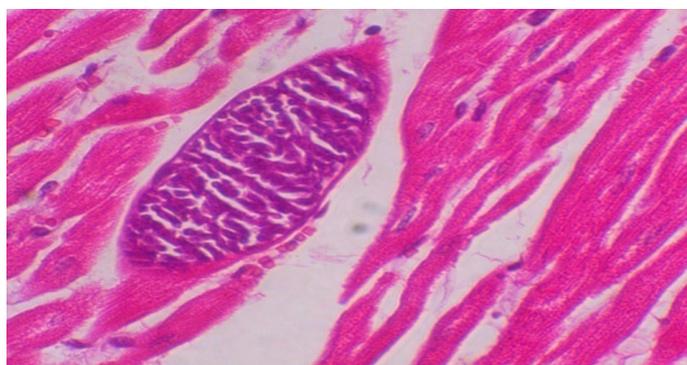


Fig. 4. Histological section representing a microscopic sarcocyst isolated from an infected buffalo's heart. The sarcocyst initiated no tissue reaction (H&E stain).

Table 4. Organ distribution of microscopic sarcocysts in slaughtered sheep.

Organ	Examined Number	Positive Number	Prevalence %
Oesophagus	200	152	76%
Heart	200	77	38.50%
Tongue	200	140	70%
Masseter muscle	200	106	53%
Skeletal muscle	200	97	48.50%

DISCUSSION

Concerning prevalence of macroscopic sarcocysts in slaughtered buffaloes and sheep, the results in Table 1, revealed that the prevalence of macroscopic sarcocysts in slaughtered buffaloes was 26.5%. Nearly, the same results were recorded in Egypt by Metwally *et al.* (2014) who recorded 25.5%, Aziz *et al.* (2017) recorded

26.9%, Mousa *et al.* (2016) recorded 23.6%, Youssef *et al.* (2013) recorded 20.33% and Nahed *et al.* (2014) recorded 30.9%. In other countries, Hamidinejat *et al.* (2009) recorded 20% in Iran and Jyothisree *et al.* (2017) recorded 22.62% in India. On the other hand, this result (26.5%) disagreed with the higher prevalence of macroscopic sarcocysts in buffaloes reported in Egypt by El-Bahy *et al.* (2019) who recorded 85.96%, El Shanawany *et al.* (2019) recorded 74% and Mousa *et al.* (2021) recorded 47.24%. In addition, this result (26.5%) disagreed with the lower prevalence of macroscopic sarcocysts in buffaloes reported in Egypt by Hussein *et al.* (2017) who recorded 3.9%, Ahmed *et al.* (2016) recorded 8.33%, Dyab *et al.* (2019) recorded 12% and El-Dakhly *et al.* (2011) recorded 6.9%. In other countries, Nicolas *et al.* (2019) recorded 4.93% in the Philippines and Oryan *et al.* (2010) recorded 3% in Iran.

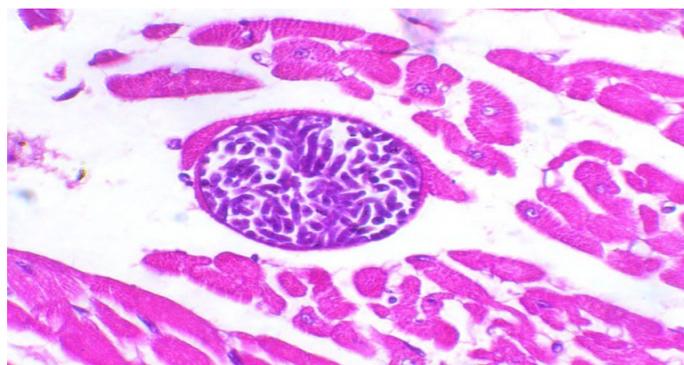


Fig. 5. Histological section representing a microscopic sarcocyst isolated from an infected sheep's heart. The sarcocyst initiated no tissue reaction (H&E stain).

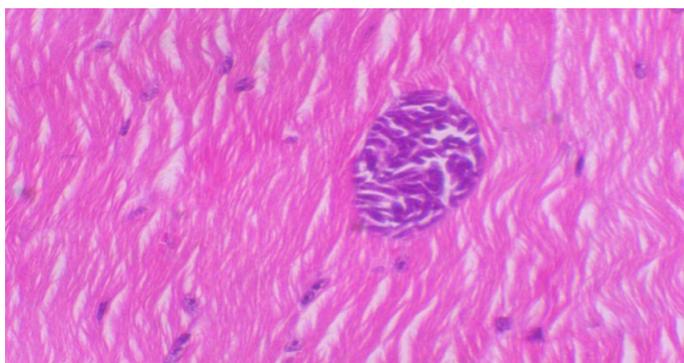


Fig. 6. Histological section representing a microscopic sarcocyst isolated from an infected sheep's tongue. The sarcocyst initiated no tissue reaction (H&E stain).

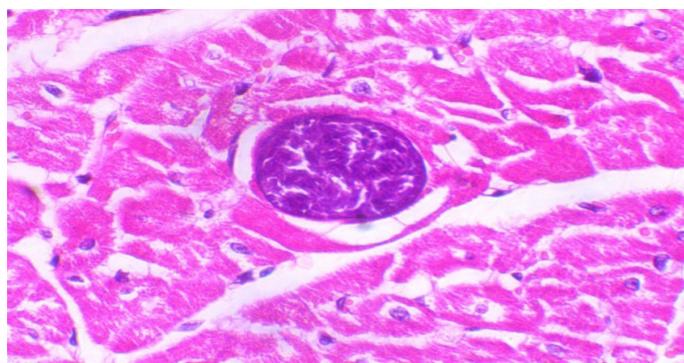


Fig.7. Histological section representing a microscopic sarcocyst isolated from an infected sheep's heart. The sarcocyst initiated no tissue reaction (H&E stain).

The obtained results in Table 1, showed that the prevalence of macroscopic sarcocysts in slaughtered sheep was 0%. Nearly, the same results were recorded by El-Morsey *et al.* (2019) who recorded 0% in El-Mahalla El-Kubra, Egypt, Hussein (2020) recorded 0% in Qena, Egypt, Bittencourt *et al.* (2016) recorded 0% in Brazil, Abdullah (2021) recorded 0% in Iraq, Dong *et al.* (2018) recorded 0% in China, Januskevicius *et al.* (2019) recorded 0%

in Lithuania, Mekibib *et al.* (2019) recorded 0% in Ethiopia. This result (0%) disagreed with El-Morsey *et al.* (2021) who recorded 13.20% in Egypt, Mahran (2009) recorded 9.9% in Shalatin, Egypt, Beyazit *et al.* (2007) recorded 24.5% in Turkey, Mirzaei Dehaghi *et al.* (2013) recorded 6% in Iran, Martínez-Navalón *et al.* (2012) recorded 12% in Spain, Pipia *et al.* (2016) recorded 23.3% in Italy, Minuzzi *et al.* (2019) recorded 7.7% in Brazil, Phythian *et al.* (2018) recorded 14.3% in Tasmanian sheep in Australia.

In the present study, the prevalence of microscopic sarcocysts in slaughtered buffaloes was 56%. Nearly, the same results were recorded by Aziz *et al.* (2017) who recorded 52.4% using the histopathological method in Sohag, Egypt, Abu-Elwafa *et al.* (2012) recorded 48.36% in Dakahlia province, Egypt, Ramakrishna *et al.* (2017) recorded 60.78% in India, Hadadzadeh *et al.* (2004) recorded 53.5% in Iran and Hamidinejat *et al.* (2009) recorded 57% using digestion method and 54% using dobs smear in Iran. This result (56%) disagreed with the lower prevalence of microscopic sarcocysts in slaughtered buffaloes obtained by Metwally *et al.* (2014) who recorded 27.7% in Assiut, Egypt, Mousa *et al.* (2016) recorded 20% in frozen buffalo meat in Alexandria, Egypt and Portella *et al.* (2021) recorded 23.75% in Brazil. Additionally, this result (56%) disagreed with the higher prevalence of microscopic sarcocysts in slaughtered buffaloes obtained by El-Dakhly *et al.* (2011) who recorded 78.9% in Beni-Suef, Egypt, Mousa *et al.* (2021) recorded 86% in Sirs-Elian, Egypt, Dar *et al.* (2017) recorded 95.5% in India, Oryan *et al.* (2010) recorded 83% in Iran and Latif *et al.* (1999) who recorded 82.9% using peptic digestion method in Iraq.

The prevalence of microscopic sarcocysts in the investigated sheep was 80.5%. Nearly, the same results were recorded by El-Morsey *et al.* (2019) who recorded 86.8% in El-Mahalla El-Kubra, Egypt, Hussein *et al.* (2018) recorded 75.96% in Qena, Egypt, Beyazit *et al.* (2007) recorded 86.5% in Turkey, Pipia *et al.* (2016) recorded 77.7% in Italy, Latif *et al.* (2015) recorded 86% in Malaysia and Berenji *et al.* (2019) recorded 81.90% in Iran. This result (80.5%) disagreed with the lower prevalence of microscopic sarcocysts in slaughtered sheep obtained by Mahran (2009) who recorded 41.26% in Shalatin, Egypt, Hussein (2020) recorded 47.27% in Qena, Egypt, Ozkayhan *et al.* (2007) recorded 47.32% in Turkey and Dong *et al.* (2018) recorded 52.51% in China. This result (80.5%) disagreed with the higher prevalence of microscopic sarcocysts in slaughtered sheep recorded by Mirzaei Dehaghi *et al.* (2013) who recorded 100% in Iran, Januskevicius *et al.* (2019) recorded 100% in Lithuania, Parandin *et al.* (2015) recorded 100% in Iran, Fukuyo *et al.* (2002) recorded 96.9% in Mongolia and Minuzzi *et al.* (2019) recorded 96.1% in Brazil. The difference in prevalence rates may be due to the different methods of diagnosis, different localities, and different management practices (Aziz *et al.*, 2017). The obtained results confirmed that Egyptian buffaloes are infected with *Sarcocystis* due to the abundance of final hosts especially dogs and cats that encourage the spreading of infection by this protozoan. Moreover, most of the Egyptian abattoirs do not have total security from the entry of stray dogs and cats which can complete the life cycle of *Sarcocystis*.

The current study recorded a higher prevalence of sarcocysts in female buffaloes (42% macroscopic sarcocysts and 79% microscopic sarcocysts) than male buffaloes (11% macroscopic sarcocysts and 33% microscopic sarcocysts). Nearly, the same results were recorded in Egypt by El-Dakhly *et al.* (2011) who recorded a higher prevalence of sarcocysts in females (17.7% macrosarcocysts and 81.2% microsarcocysts) than in males (1.3% macrosarcocysts and 74.5% microsarcocysts), Ibrahim *et al.* (2018) recorded a higher prevalence of sarcocysts in females (37% macrosarcocysts and 49.6% microsarcocysts) than males (25.8% macrosarcocysts and 36.2% microsarcocysts), Mousa *et al.* (2021) recorded a higher prevalence of sarcocysts in females (63.39% macrosarcocysts and 92% microsarcocysts) than males (3.64% macrosarcocysts and 80% microsarcocysts) and Jyothisree *et al.* (2017) recorded a higher prevalence of sarcocysts in females (39.51% macrosarcocysts and 45.83% microsarcocysts) than males (13.4% macrosarcocysts and 42.69% microsarcocysts) in

India. The low percentage of the infected males may be attributed to the animal management system in Egypt, as most of the males are kept only for the fattening system and are slaughtered around 2 years old, while females are kept for long times for milk production (El Shanawany *et al.*, 2019).

In this study, the prevalence of microscopic sarcocysts in female sheep (93%) was higher than male sheep (68%). Nearly, the same results were recorded by Hussein (2020) who recorded a higher prevalence of microscopic sarcocysts in sheep females (64.70%) than sheep males (44.08%) in Qena, Egypt, Ibrahim *et al.* (2018) recorded a higher prevalence of microscopic sarcocysts in sheep females (24.8%) than sheep males (16.6%) in Cairo, Egypt, Abuelwafa *et al.* (2016) recorded a higher prevalence of microscopic sarcocysts in sheep females (99%) than sheep males (94.55%) in Dakahlia province, Egypt.

Results from the present study revealed a higher prevalence of sarcocysts in old buffaloes (42% macroscopic sarcocysts and 79% microscopic sarcocysts) than in young buffaloes (11% macroscopic sarcocysts and 33% microscopic sarcocysts). Nearly, the same results were recorded by Ibrahim *et al.* (2018) who recorded a higher prevalence of sarcocysts in old buffaloes (48.6% macrosarcocysts and 63.2% microsarcocysts) than in young buffaloes (41.2% macrosarcocysts and 53.8% microsarcocysts) in Cairo, Egypt, El-Dakhly *et al.* (2011) recorded a higher prevalence of sarcocysts in old buffaloes (17.7% macrosarcocysts and 81.2% microsarcocysts) than young buffaloes (1.3% macrosarcocysts and 74.5% microsarcocysts) in Beni-Suef, Egypt and Jyothisree *et al.* (2017) recorded a higher prevalence of sarcocysts in old buffaloes (39.51% macrosarcocysts and 45.83% microsarcocysts) than young buffaloes (13.4% macrosarcocysts and 42.69% microsarcocysts) in India. The correlation between the age and the increased infection rate may be attributed to repeated exposure to *Sarcocystis* infection, which results in sarcocysts accumulation gradually inside muscles (Taib *et al.*, 2016).

The results in Table 2, revealed a higher prevalence of microscopic sarcocysts in old sheep (93%) than in young sheep (68%). Nearly, the same results were recorded by Abuelwafa *et al.* (2016) who recorded a higher prevalence of microscopic sarcocysts in old sheep (98.5%) than in young sheep (86.57%) in Dakahlia province, Egypt, Hussein (2020) recorded a higher prevalence of microscopic sarcocysts in old sheep (59.42%) than young sheep (26.82%) in Qena, Egypt, Ibrahim *et al.* (2018) recorded a higher prevalence of microscopic sarcocysts in old sheep (27.2%) than young sheep (17.8%) in Cairo, Egypt and Ozkayhan *et al.* (2007) who recorded a higher prevalence of microscopic sarcocysts in old sheep (59.25%) than young sheep (16.12%) in Turkey.

Concerning organ distribution of sarcocysts in slaughtered buffaloes, the most infected organ with microsarcocysts was the oesophagus (53.5%) followed by the tongue (45.5%), masseter muscle (41%), skeletal muscle (32%) and the least infected organ was the heart (24%). Nearly, the same results were recorded by El-Dakhly *et al.* (2011) who recorded the highest prevalence of microsarcocysts in the oesophagus (74.1% in old buffaloes and 68% in young buffaloes) followed by the tongue (15.3% in old buffaloes and 6.5% in young buffaloes) and the least prevalence in the heart (7.1% in old buffaloes and 3.9% in young buffaloes) using the histological method in Beni-Suef, Egypt, Jyothisree *et al.* (2017) recorded the highest prevalence of microsarcocysts in the oesophagus (51.82%) followed by the tongue (47.44%) and heart (29.92%) in India, Nahed *et al.* (2014) recorded the highest prevalence of microsarcocysts in the oesophagus (68.96%) in Egypt and Ibrahim *et al.* (2018) recorded the highest prevalence of sarcocysts in the oesophagus followed by the tongue and finally the heart. On the contrary, Oryan *et al.* (2010) recorded the highest prevalence of microsarcocysts in masseter muscle (57%) followed by the tongue (44%) in Iran, Latif *et al.* (2013) recorded the highest prevalence of microsarcocysts in the heart (66.7%) followed by oesophagus (50%) in Malaysia, Dar *et al.* (2017) recorded that the tongue was the most infected organ by microsarcocysts (87.40%) followed by oesophagus (83.60%) in India and Mousa *et al.* (2021) recorded that the tongue was the most

infected organ by microsarcocysts (46%) in Sirs-Elian, Egypt.

Furthermore, the most infected organ with microsarcocysts in sheep was the oesophagus (76%) followed by the tongue (70%), masseter muscle (53%), skeletal muscle (48.5%) and the least infected organ was the heart (38.5%). Nearly, the same results were obtained by Nageib and kuraa (2018) who recorded the highest prevalence of microscopic sarcocysts in the oesophagus (71%) in Assiut, Egypt, El-Morsey *et al.* (2019) recorded the highest prevalence of microscopic sarcocysts in the oesophagus (62.3%) in Egypt, Hu *et al.* (2017) recorded the highest prevalence of microscopic sarcocysts in the oesophagus (84.9%) in China and Abdullah (2021) recorded the highest prevalence of microscopic sarcocysts in the oesophagus (95%) in Iraq. In contrast, Bittencourt *et al.* (2016) recorded that tongue was the most infected organ (86.7%) using the squash method in Brazil, Fukuyo *et al.* (2002) recorded that tongue was the most infected organ (100%) in Mongolia, Latif *et al.* (2015) recorded that skeletal muscle was the most infected organ (64.9%) in Malaysia.

CONCLUSION

This study reports the presence of *Sarcocystis* species in buffaloes and sheep slaughtered at Tanta abattoir, Egypt. Therefore, meat should be cooked thoroughly or frozen before usage to prevent zoonotic foodborne transmission into human consumers. The obtained results confirmed that Egyptian buffaloes and sheep are infected with *Sarcocystis* species due to the abundance of final hosts, especially dogs and cats that encourage the spreading of infection by this protozoan. Strict control measures should be applied to stray dogs and cats in developing countries especially Egypt, as they play serious roles in transmitting infection with different species of *Sarcocystis* to buffaloes and sheep. Therefore, the concerned authority should make efforts for proper meat inspection procedures and combat street dogs and cats.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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